REMARKS

Claims 1-4 and 32-36 are in this application.

The Examiner has objected to the specification because of references to previous studies. As stated in the Response to the Official Action of June 6, 2001 the previous studies are applicants' own studies and are disclosed in U.S. Patent No. 6,156,725. Pages 2, 3 and 5 of this application have been amended to include that the previous studies are the studies described in US Patent No. 6,156,725.

The typographical error on page 1 has been corrected.

Therefore, it is respectfully requested that this objection be withdrawn.

The Examiner rejects claims 1-4 and 32-36 because of the use of Dxg in the claims. This rejection is respectfully traversed. Some sequences include Aib, others include Dxg as a cyclic or acyclic dialkylated glycine and others contain both. One skilled in the art would understand the metes and bounds of the claims that contain Dxg. However, to expedite prosecution of this application Dxg has been replaced by Deg (α,α -diethyl glycine) in claims 1, 2, and 33-36. Deg is a acyclic dialkylated glycine and is diethyl glycine. A new biological sequence listing is also attached. Applicants preserve all rights to file one or more continuation or divisional applications for peptides that include other cyclic or acyclic dialkylated glycines. Therefore, it is respectfully requested that this rejection be withdrawn.

The Examiner has rejected claims 1-4 and 32 34 under 35 USC 103(a) over Gozes (US Patent 5,217,853) in view of Spatola (Chemistry

and Biochemistry of Amino Acids, Peptides and Proteins, Chapter 5, page 271, 1983). It is not clear from the Official Action whether claims 32 and 34 are rejected or claims 32, 33 and 34. Applicants respectfully traverse this rejection.

The examiner states that the primary reference of Gozes *et al* teaches that VIP antagonists are known and used to treat cancer. Further the secondary reference of Spatola et al teaches to modify peptides of the primary reference of Gozes et al to obtain stability and resistance to enzymatic degradation. The Examiner relies on In re Betz "the test of obviousness is not express suggestion of the claimed invention in any and all of the references but rather what the references taken collectively would suggest to those of ordinary skill in the art presumed to be familiar with them." However, it is respectfully argued that there is no combination of these references that makes obvious the claimed invention. The consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that the process should be carried out and would have a reasonable likelihood of success, viewed in the light of the prior art. Bothe the suggestion and the expectation of success must be founded in the prior art, not in the applicant's disclosure (In re O'OFarrell 7 USPQ2d 1673 (Fed. Cir 1988).

As explained in Spatola there is no reasonable expectation of success that replacing one amino acid with another with achieve enhanced activity. For example, on page 279 Spatola states that N-methylation at the 7 position residue enhances the activity of some analogs but there is a lack of additivity observed in multiply modified analogs and N-methylation in certain modified LH-RH hormones caused a drop in potency. On page 280 it is stated that N-methylated bradykin analogs at positions 1, 4, 5, 8, and 9 were prepared but only the N-methylated at position 1 was equipotent. However, methylated substance P analogs were active. N-methylation of a Phe 11 residue in a neurotenin analog gave a compound with reduced potency. On page 281 it is stated that potency and selectivity were found to vary with bond placement. It is stated that peptides might introduce quite different conformational ramifications lends further support to the need to document all chemical and spectral parameters with any new backbone replacement.

In regard to the use of D-amino acids it is stated on page 285 of Spatola that "among the most commonly modified peptide, only about 10% of the total possible monosubstituted structures yield compounds with retained or enhanced biological potencies."

As summarized on page 343 of Spatola "Generalizations regarding the efficacy of a particular modification are probably not yet justified nor justifiable. While the degree of isosterism of modifications certainly varies, the constantly changing importance of steric, electronic, geometric and lipophilic parameters makes it virtually impossible to cite one modification as inherently superior to another and it is likely each of the various types described or often combinations of these modifications will prove efficacious, depending on the particular patent peptide chose."

There is no prior art including Spatola et al, wherein "Dxg"(α,α-dialkylated amino acids) is incorporated in the peptides to make it more stable and resistant to enzymatic degradation. Further, the rendering of the enzymatic stability to the peptide by introduction of "Dxg" (e.g., Deg) in the claimed peptide sequences is a totally unexpected result, which was not motivated by Spatola's teachings since Spatola et al. have not used "Dxg" in their disclosed invention.

Therefore since there is no reasonable expectation of success in the combination of Gozes and Spatola, and it is respectfully requested that this rejection be withdrawn.

The Examiner has rejected claims 1-4 and 32-36 under 35 USC 112, first paragraph as not being enabled. Applicants respectfully traverse that rejection.

The following working examples provide evidence, which shows that the claimed peptides (DT-19 in this case SEQ ID NO: 9) is useful when administered as an active ingredient in a pharmaceutical composition at a therapeutically effective dose to treat cancer of colon in a mammal. It also evidences the method of treatment thereof as recited in claims 1-4 and 32-36. Further, the examples when viewed in the context of the application

provide sufficient guidance to enable a person skilled in the art to make and use the claimed invention without undue experimentation.

The examples also describe a therapeutically effective pharmaceutical composition containing an individual peptide for in vivo treatment of colon cancer in a mammal. Further, the dosage amount and the pharmaceutical composition have been disclosed.

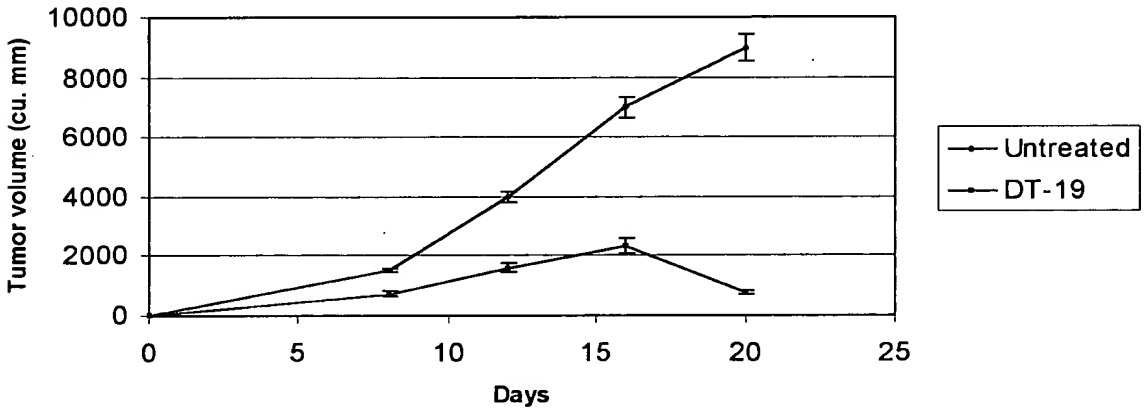
Example

A method for treating cancer in an animal using DT-19

The antitumor activity of DT-19 was studied in human colon adenocarcinoma (PTC) xenografts in nude mice. PTC tumor xenografts were grown in Balb/c athymic mice by subcutaneous inoculation of a single cell suspension of PTC cells (15 X 10^6 cells/100 mL). The tumor bearing animals were divided into 2 groups of three animals each including one group comprising untreated control animals. Treatment with DT-19 was initiated when the average tumor volumes, as measured using a vernier caliper, were between 1.3 cm^3. Solutions of SEQ ID: was prepared at a concentration of 126mg/ml and intravenously administered to the assigned group of tumor bearing animals at a dose of 12.5 mg/100 mL twice a day so that the total dose of 25mg/day was administered to each animal. The treatment was continued for a period of 14 days.

The antitumor activity of the compounds was monitored by measuring tumor volumes every fourth day using the formula W*W*L*0.4 (W = smaller diameter, L = larger diameter). The percentage inhibition of tumor growth was calculated using the formula (1- tumor volume-treated / tumor volume-control) * 100. Table 1 shows the tumor volumes of individual animals measured till day 20 post-inoculation. Figure 1 shows the tumor kinetics till day 20 in the treated and untreated animals. DT-19 showed a significant antitumor activity on PTC xenografts. The percentage inhibition of tumor growth caused by DT-19 as compared to controls on day 20 was 91.7%.

In vivo antitumor activity of DT19 on colon xenograft



Example

Pharmaceutical composition and therapeutic dose of claimed peptides

An example within the scope of the invention comprises of peptides DT-11 to DT-19. The molar concentration of each of the peptides where it is expected to be active ranges from 10^{-7} M to 10^{-10} M. However, it is expected that these peptides would be effective if the concentration of each ranged from approximately 10^{-6} M to approximately 10^{-12} M.

A formulation of each of these peptides for in vitro use may be prepared in the following way. A stock solution of each of the peptide analogs is prepared with a pH of approximately 7.0 to approximately 7.4. Although

sterile phosphate buffered saline was used to prepare the stock solutions for the testing described below, other diluents may be used such as RPMI 1640, buffered saline, isotonic NaCl, Ringer's solution, water (for injection), distilled water, polyethylene glycol (neat or in water), 2% Tween in water, dimethylsulfoxide to 50% in water, propylene glycol (neat or in water), balanced salt solution, glycerol, and other conventional fluids that are suitable for intravenous administration. To obtain a pH in the range of approximately 7.0 to 7.4 for each stock solution, the pH can be adjusted by using 1N HCL for lowering the pH or 1N NaOH for raising the pH, although other conventional agents for adjusting the pH can be used. The concentration of the peptide analog is approximately 10^{-3} M. This is further diluted using the above-mentioned diluents to give a final concentration of 10^{-8} M. In one exemplary embodiment, the pH of the peptide solution may range from approximately 7.0 to approximately 7.4. To obtain a pH in this range, the pH can be adjusted by using 1N HCL for lowering the pH or 1N NaOH for raising the pH, although other conventional agents for adjusting the pH can be used.

The methods of this invention comprise, consist of, or consist essentially of: administering systemically to the mammal a therapeutically effective quantity of any of the mentioned peptides DT-11 to DT-19. An effective dose ranges form 15 to 170 ug (preferably 25 to 40 ug) of the peptides per kg of the body weight of the mammal, with the dose dependent on the effects sought, the manner of administration, the peptides selected, and the cancer being treated. Systemic administration refers to oral, rectal, nasal, transdermal, and parental (i.e., intramuscular, intravenous and subcutaneous). In accordance with good clinical practice, it is preferred to administer the composition at a dose that will produce anticancer effects without causing undue harmful side effects. The composition may be administered either alone or as a mixture with other therapeutic agents.

The composition may optionally and preferably contain pharmaceutically acceptable diluents, excipients, solvents, binders, stabilizers, and the like. Such diluents may include: RPMI 1649, buffered saline, isotonic NaCl, Ringer's solution, water, distilled water, polyethylene glycol (neat or in water), 2% Tween in water, dimethylsulfoxide to 50% in water, propylene glycol (neat or in water), phosphate buffered saline,

balanced salt solution, glycerol, and other conventional fluids that are suitable for intravenous administration. Pharmaceutical composition which provide from about 10 to 2000ug of the composition per unit dose are preferred and are conventionally prepared as tablets, lozenges, capsules, powders, aqueous or oily suspension, syrups, elixirs, and aqueous solutions. The nature of the pharmaceutical composition employed will, of course, depend on the desired route of administration.

Formulation of a dose of VIP analogs DT-11 to DT-19 for in vivo experiments:

A dose of the peptide formulation was prepared in the following way. A stock solution of peptide analog was first prepared using sterile phosphate buffered saline with an approximate pH of 7.4. The weight of the peptide analog per dose is approximately 1 to 100 mg. The volume of this solution was made up with sterile RPMI1640 to approximately 100ul.

The use of cell lines to test for anticancer activity is well know in the art. The National Cancer Institute, Bethesda, Maryland subjects all of its potential anticancer molecules showing promising activity *in vitro* on cell lines representative of *in vivo* models Br J Cancer. 2001 May 18; 84(10):1289-90 (Relationships between drug activity in NCI preclinical in vitro and vivo models and early clinical trials); Semin Oncol 1992 Dec; 19(6):622-38 (The National Cancer Institute; Cancer drug discovery and development program), Japanese J Antibiot 1977 Dec; 30 Suppl:35-40 (Antitumor screening procedures of the National Cancer Institute).

A database search of the National Library of Medicine was carried out to determine the relevance of cell lines used by the applicants for determining the anticancer activity of the peptides. HT29 (human colon) showed 1083 "hits" when searched with reference to cancer and the other human cancer cell lines used also showed a large number of hits (832 for A549, 727 for MOLT-4, 475 for DU145, 199 for HBI, 196 for SW620 and 77 for PA-1). This clearly shows the extensive use of these cell lines in cancer research. Further, it is a common and standard practice and norm for testing molecules showing promising anticancer activity *in vitro* to be

tested in *in vivo* models.

Peptides of this invention were tested in the HT-29, SW620, PTC, PA-1,A549, HBL100, MOLT-4 and DU145 which represent colon, ovary, lung, breast, leukemia and prostate cancer. As explained above positive results in these tests will lead to further testing in vivo. The protocol results are given in Example 1 see Table 1 where SEQ IDS NO:2-9 (corresponding to DT-11 to DT-19 were tested).

Since the applicants have explained the protocol for testing the peptides of this invention *in vitro* and have established that these in vitro screening methods are used by those of skill in the art to determine anticancer activity and that positive results in these studies can lead to further in vivo testing and have shown how to use the claimed peptides in these assays, it is respectfully requested that this rejection be withdrawn.

Applicants submit that the present application is in condition for allowance and favorable consideration is respectfully requested.

Respectfully submitted,

Janet I. Cord
c/o Ladas & Parry
26 West 61st Street
New York, New York 10023
Reg. No. 33,778 (212) 708-1935

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In the Specification

Please replace Paragraph 5 on page 1 with the following:

Page 1, Paragraph 5. In U.S. Patent Application 08/727,679 now U.S. Patent 6,156,725, we have described the role of neuropeptides in cancer. High affinity and moderate affinity receptors for vasoactive intestinal peptide and somastostatin, high affinity receptors for bombesin and moderate affinity receptors for substance P were demonstrated on human colon adenocarcinoma cells. It was further demonstrated that peptide analogs to the above neuropeptides could actively and selectively induce cell death in the cancer cells. A formulation of peptide combination termed [MuJ-&] MuJ-7 has also been described which causes tumor regression in xenotransplated nude mice. The individual constituent peptides of MuJ-7 were demonstrated to have anticancer activity.

Please replace paragraph 4 on page 2 with the following:

The VIP receptor binding inhibitor VIP_2 (Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys) (SEQ ID NO:1) has been shown in our previous studies incorporated in U.S. Patent 6,156,725 to be a selective cytotoxic peptide for cancer cells having receptors for vasoactive intestinal peptide. Novel peptides that have conformational constraints and resist enzymatic degradation are formed by replacing any of the amino acids of the sequence Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys with Dxg. Dxg represents cyclic and acyclic dialkylate glycines where the cyclic ring is C_3-C_8 ring and the number of carbon atoms in the alkyl group is from 1 to 6 (methyl to hexyl). Examples are Aib, MeLeu, Di-ethyglycine and its higher homologs, and 1-amino cycloalkane carboxylic acids. Aib represents α-amino-isobutyric acid. Deg represents di-ethyglycine.

Please replace paragraph 1 on page 3 with the following:

Novel peptides include:

DT-11 Aib-Met-Tyr-Pro-Thr-Tyr-Aib-Lys-OH (SEQ ID NO:2)

DT-12 D-Leu-Met-Tyr-Pro-Thr-Tyr-Aib-Lys-OH (SEQ ID NO:3)

DT-13 Leu-Met-Tyr-Pro-Thr-D-Tyr-Leu-Lys-OH (SEQ ID NO:4)

DT-14 Leu-Met-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH (SEQ ID NO:5)

DT-15 Leu-Met-D-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH (SEQ ID NO:6)

DT-16 D-Leu-Met-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH (SEQ ID NO:7)

DT-18 Aib-Met-Tyr-Pro-Thr-Tyr-[Dxg] Deg-Lys-OH (SEQ ID NO:8)

DT-19 D-Leu-Met-Tyr-Pro-Thr-Tyr-[Dxg] Deg-Lys-OH (SEQ ID NO:9)

where [Dxg] Deg and Aib are as defined above.

Please replace paragraph 2 on page 3 with the following:

The bombesing antagonist BOM_1 (D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NHEt) (SEQ ID NO:10) has been shown in our previous studies incorporated in U.S. Patent 6,156,725 to be a selective cytotoxic peptide for cancer cells having receptors for bombesin. Novel peptides that have conformational constraints and resist enzymatic degradation are formed by replacing any of the amino acids of the sequence D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NHEt (SEQ ID NO:10) with Dxg where Dxg is defined above. Leucine may be replaced with isoleucine and tryptophan may be replaced by D-Tryptophan.

Please replace paragraph 1 on page 4 with the following:

The Substance P analog (D-Arg-Pro-Lys-Pro-D-Phe-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH_2 (SEQ ID NO:18)) has been shown in our previous studies

incorporated in U.S. Patent 6,156,725 to be a selective cyctotoxic peptide for cancer cells having receptors for Substance P. Novel peptides that have conformational constraints and resist enzymatic degradation are formed by replacing any of the amino acids of the sequence D-Arg-Pro-Lys-Pro-D-Phe-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH_2 (SEQ ID NO:18) with Dxg or Aib. Dxg and Aib are as defined above. Analogs may be 5 to 11 amino acids.

Please replace paragraph 2 on page 4 with the following:

Novel peptides include:

DT-31 Aib-Met-Gln-Trp-Phe-Aib-NH_2 (SEQ ID NO:19)

DT-32 [Dxg] Deg-Met-Gln-Trp-Phe-Aib-NH_2 (SEQ ID NO:20)

DT-33 D-Leu-Met-Gln-Trp-Phe-Aib-NH_2 (SEQ ID NO:21)

DT-34 D-Arg-Pro-Lys-Pro-Aib-Gln-D-Trp-Phe-D-Trp-Aib-Leu-NH_2 (SEQ ID NO:22)

DT-35 Arg-Pro-Aib-Pro-D-Phe-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH_2 (SEQ ID NO:23)

where [Dxg] Deg and Aib are as defined above.

Please replace paragraph 3 on page 4 with the following:

The somatostatin analog (Ala-Gly-Cys-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Ser-D-Cys (disulfide brideges: 3-14) (SEQ ID NO:24) has been shown in our previous studies incorporated in U.S. Patent 6,156,725 to be a selective cytotoxic peptide for cancer cells having receptors for Somatostatin. Novel peptides that have conformational constraints and resist enzymatic degradation are formed by replacing any of the amino acids of the sequence (Ala-Gly-Cys-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Ser-D-Cys (disulfide bridges:3-14) (SEQ ID NO:24) with Dxg or Aib where Dxg and Aib are as described above.

Please replace paragraph 2 on page 5 with the following:

The somatostatin analog (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2) (SEQ ID NO:26) has been shown in our previous studies incorporated in U.S. Patent 6,156,725 to be a selective cytotoxic peptide for cancer cells having receptors for Somatostatin. Novel peptides that have conformational constraints and resist enzymatic degradation are formed by replacing any of the amino acids of the sequence D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (SEQ ID NO:26) with Dxg or Aib where Dxg and Aib are as defined above.

In the Claims

Claim 1 (Twice Amended) A peptide of the sequence Leu^1-Met^2-Tyr^3-Pro^4-Thr^5-Tyr^6-Leu^7-Lys^8 (SEQ ID NO:1) wherein at least one of the amino acids at positions 1-8 is replaced by Deg [Dxg] [SEQ ID NO:1].

Claim 2 (Once Amended) A peptide having the sequence selected from the group consisting of:

Aib-Met-Tyr-Pro-Thr-Tyr-Aib-Lys-OH (SEQ ID NO: 2);
D-Leu-Met-Tyr-Pro-Thr-Tyr-Aib-Lys-OH (SEQ ID NO:3);
Leu-Met-Tyr-Pro-Thr-D-Tyr-Leu-Lys-OH (SEQ ID NO: 4);
Leu-Met-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH (SEQ ID NO: 5);
Leu-Met-D-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH (SEQ ID NO: 6);
D-Leu-Met-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH (SEQ ID NO: 7);
Aib-Met-Tyr-Pro-Thr-Tyr-[Dxg] Deg-Lys-OH (SEQ ID NO: 8); and
D-Leu-Met-Tyr-Pro-Thr-Tyr-[Dxg] Deg-Lys-OH (SEQ ID NO: 9).

Claim 33 (Amended) A method for treating breast cancer comprising administering an effective amount of a peptide selected from the group consisting of:

Aib-Met-Tyr-Pro-Thr-Tyr-Aib-Lys-OH (SEQ ID NO:2);
D-Leu-Met-Tyr-Pro-Thr-Tyr-Aib-Lys-OH (SEQ ID NO:3);
Leu-Met-Tyr-Pro-Thr-D-Tyr-Leu-Lys-OH (SEQ ID NO:4);
Leu-Met-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH (SEQ ID NO:5);
Leu-Met-D-Tyr-Pro-Thy-Tyr-D-Leu-Lys-OH (SEQ ID NO:6);
Aib-Met-Tyr-Pro-Thr-Tyr-[Dxg]Deg -Lys-OH (SEQ ID NO:8); and
D-Leu-Met-Tyr-Pro-Thr-Tyr-[Dxg] Deg-Lys-OH (SEQ ID NO:9).

Claim 34 (Amended) A method for treating breast cancer comprising administering an effective amount of a peptide selected from the group consisting of:

Leu-Met-D-Tyr-Pro-Thy-Tyr-D-Leu-Lys-OH (SEQ ID NO:6);
D-Leu-Met-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH (SEQ ID NO:7);
Aib-Met-Tyr-Pro-Thr-Tyr-[Dxg]Deg -Lys-OH (SEQ ID NO:8); and
D-Leu-Met-Tyr-Pro-Thr-Tyr-[Dxg] Deg-Lys-OH (SEQ ID NO:9).

Claim 35 (Amended) A method for treating colon cancer comprising administering a peptide selected from the group consisting of:

Leu-Met-D-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH (SEQ ID NO: 6);
D-Leu-Met-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH (SEQ ID NO: 7);
Aib-Met-Tyr-Pro-Thr-Tyr-[Dxg] Deg-Lys-OH (SEQ ID NO: 8); and
D-Leu-Met-Tyr-Pro-Thr-Tyr-[Dxg] Deg-Lys-OH (SEQ ID NO: 9).

Claim 36 (Amended) A method for treating prostate cancer comprising administering a peptide selected from the group consisting of:

D-Leu-Met-Tyr-Pro-Thr-Tyr-Aib-Lys-OH (SEQ ID NO:3);
Aib-Met-Tyr-Pro-Thr-Tyr-[Dxg]Deg -Lys-OH (SEQ ID NO:8); and
D-Leu-Met-Tyr-Pro-Thr-Tyr-[Dxg] Deg-Lys-OH (SEQ ID NO:9).